A / RE

PATENT
Attorney Docket No. 03804.1129-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of:
U.S. Patent No. 5,837,531

Issued: November 17, 1998

Inventors: Jean-Francois DEDIEU et al.

Group Art Unit: Unassigned

Reissue Serial No.: Unassigned

Examiner: Unassigned

Reissue Application Filed: August 31, 2000

For: RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS

REISSUE PATENT APPLICATION TRANSMITTAL

ATTN.: BOX REISSUE
Assistant Commissioner for Patents
Washington, D.C. 20231

APPLICATION FOR REISSUE OF: ☒ Utility Patent ☐ Design Patent

1. ☒ Fee Transmittal Form

2. ☒ Specification and Claims (amended, if appropriate)

3. ☒ Drawing(s) (proposed amendments, if appropriate)

4. ☒ Reissue Oath/Declaration (3 original copies) (37 C.F.R. § 1.175)

5. Original U.S. Patent
   - ☒ Offer to Surrender Original Patent
   - ☐ Ribboned Original Patent
   - ☐ Affidavit/Declaration of Loss

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6. Original U.S. Patent currently assigned?

☒ Yes ☐ No

(If Yes, check applicable box(es))

☒ Written Consent of all Assignees

☒ 37 C.F.R. § 3.73(b) Statement

☒ Power of Attorney

7. ☒ Foreign Priority Claim (35 U.S.C. § 119)

8. ☒ Information Disclosure Statement (IDS) / PTO 1449

☐ Copies of IDS Citations

9. ☐ English Translation of Reissue Oath/Declaration

10. ☐ Small Entity Statement(s)

☐ Statement filed in prior application, status still proper and desired

11. ☒ Preliminary Amendment

12. ☐ Also enclosed is: U.S. Patent No. 5,837,531 in single column format

If there are any fees due under 37 C.F.R. §§ 1.16 or 1.17 which are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: August 31, 2000

By: _____
Timothy B. Donaldson
Reg. No. 43,592

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PATENT
Attorney Docket No. 03804.1129-00000
RPR Docket No. EX93019-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Sir:

CONSENT OF ASSIGNEE, OFFER TO SURRENDER ORIGINAL PATENT, POWER OF ATTORNEY, AND STATEMENT UNDER 37 C.F.R. § 3.73(b)

U.S. Patent No. 5,837,531 is assigned to Aventis Pharma S.A., formerly Rhône-Poulenc Rorer, S.A., as assignee of the entire right, title and interest in said patent by virtue of an assignment from Jean-Francois DEDIEU, Aude LE ROUX, and Michel PERRICAUDET ("the Inventors") to Rhône-Poulenc Rorer, S.A. recorded at Reel 007974, Frame 0407. Copies of these papers are attached.

Aventis Pharma S.A., formerly Rhône-Poulenc Rorer, S.A., hereby consents to the accompanying application for reissue of U.S. Patent 5,837,531.

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Aventis Pharma S.A., formerly Rhône-Poulenc Rorer, S.A., hereby offers to surrender U.S. Patent 5,837,531, when the reissue application is otherwise in condition for allowance.

Aventis Pharma S.A., formerly Rhône-Poulenc Rorer, S.A., hereby grants power of attorney to **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.,** Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; Roger D. Taylor, Reg. No. 28,992; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr.,

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Finnegan, Henderson, Farabow,
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The undersigned, whose title is supplied below, is empowered to sign on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 5, 2000
Date

Signature

ROXANE DERNONCOUR
Name

AVENTIS PHARMA S.A.
PATENT ATTORNEY
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Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

This amendment is being submitted concurrently with filing a reissue application for the above patent. Prior to examination of the reissue application on the merits, please enter the following amendment:

IN THE CLAIMS:

Please amend the claims as follows:

1. (Amended) A replication defective recombinant adenovirus comprising a heterologous DNA sequence under the control of an expression signal which is inducible by [the] an Epstein-Barr virus (EBV) antigen or by a papilloma virus antigen.

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2. (Amended) An adenovirus according to claim 1, wherein the [expression signal is activated by the] EBV antigen is EBNA 1 [antigen].

REMARKS

U.S. Patent No. 5,837,531 ("the '531 patent") issued on November 17, 1998, with claims 1-16. A reissue application is being filed pursuant to 35 U.S.C. § 251 to correct a potential ambiguity in the claimed subject matter that occurred through error without any deceptive intent. This preliminary amendment is being filed concurrently with the reissue application.

Claims 1-16 are pending in this reissue application. Claims 1 and 2 have been amended to clarify the claimed subject matter. In accordance with 37 C.F.R. § 1.121(b)(2)(iii), support for the amendments to claims 1 and 2 is found, for example, at column 4, lines 32-58. Thus, no new matter has been added.

Claim 2 of the '531 patent has a potential antecedent basis problem, which could render the claim vague and indefinite under 35 U.S.C. § 112, second paragraph. Claim 2 depends from claim 1 and recites that "the expression signal is activated by the EBNA 1 antigen." Claim 1 recites that the expression signal "is inducible by the Epstein-Barr virus (EBV) or a papilloma virus antigen." Thus, claim 2 lacks direct antecedent basis for "the EBNA 1 antigen." To provide proper antecedent basis and eliminate this potential ambiguity, claim 1 was amended to recite that the expression signal is inducible by "an Epstein-Barr virus antigen" rather than by

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"the Epstein-Barr virus," as currently recited. In addition, claim 2 was amended to recite "[a]n adenovirus according to claim 1, wherein the Epstein-Barr virus antigen is EBNA1."

CONCLUSION

In view of the foregoing amendments and remarks, the patent owner respectfully requests reconsideration and reexamination of this application and timely allowance of the pending claims.

If there are any fees due in connection with the filing of this Preliminary Amendment not already accounted for, please charge the fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
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By: _____
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Dated: August 31, 2000

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RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS

This application is a national phase application under 35 U.S.C. 371 of PCT/FR 94/01284, filed Nov. 07, 1994.

The present invention relates to recombinant vectors of viral origin and to their use for the treatment of cancers. More particularly, it relates to recombinant adenoviruses containing a heterologous DNA sequence under the control of expression signals which are active specifically in tumour cells. The invention also relates to the preparation of these vectors, to the pharmaceutical compositions containing them and to their use in gene therapy.

Gene therapy consists in correcting a deficiency or an abnormality (mutation, aberrant expression and the like) by the introduction of a genetic information into the cell or the affected organ. This genetic information can be introduced either in vitro into a cell extracted from the organ, the modified cell then being reintroduced into the body, or directly in vivo into the appropriate tissue. Various techniques have been described for the introduction of this genetic information, amongst which are various transfection techniques involving complexes of DNA and DEAE-dextran (Pagano et al., J. Virol. 1 (1967) 891), of DNA and nuclear proteins (Kaneda et al., Science 243 (1989) 375), of DNA and lipids (Felgner et al., PNAS 84 (1987) 7413), the use of liposomes (Fraley et al., J. Biol. Chem. 255 (1980) 10431), and the like. More recently, the use of viruses as vectors for the transfer of genes appeared as a promising alternative to these physical transfection techniques. In this respect, various viruses have been tested for their capacity to infect certain cell populations, in particular retroviruses (RSV, HMS, MMS and the like), HSV virus, adeno-associated viruses, and adenoviruses.

Numerous applications of gene therapy are under study, such as genetic diseases (myopathy, cystic fibrosis, SCID, and the like), pathologies of the central nervous system (Alzheimer, Parkinson, and the like), cardiovascular diseases (haemophilia, atherosclerosis), AIDS or cancers. More particularly, as regards the treatment of cancers, various strategies have been proposed in-the prior art. Thus, Application EP 259 212 describes the preparation of vaccines intended for the treatment of cancers, comprising a modified virus capable of expressing an antigen specific for a tumour, permitting an immune response to be generated against these cells. Moreover, Application WO91/15580 describes the construction of retroviruses containing a gene encoding a ribozyme, whose expression in cell culture can make it possible to destroy an mRNA of an oncogene. It is also known from Application WO93/10814 to use vectors expressing immunogenic, non-tumorigenic forms of cellular oncogenes involved in the development of cancers. Application WO93/02556 finally describes the use of cells removed from the tumour, which are genetically modified ex vivo by the introduction of a toxic gene, then readministered to the patient. However, this approach requires surgical steps, and furthermore, the stability of the toxic gene in the cell transformed ex vivo is not established.

Consequently, although valuable results have been obtained, the constructs described in the prior art do not make it possible to satisfactorily solve some difficulties, and especially the precise screening of the cells to be treated. Thus, it has been proposed to use recombinant retroviruses as vectors for the transfer of therapeutic genes. Indeed, these viruses are capable of infecting only the cells which divide. However, the use of this type of vector does not make it possible to screen, with sufficient selectivity, the tumour

cells. Furthermore, these viruses cannot be obtained at very high titres, thereby limiting the therapeutic efficacy. It has moreover been proposed to directly administer the gene into the tumour. Here again, the risks of diffusion to the surrounding cells are not excluded. For this reason, it has been proposed to modify the host specificity of the viruses used, by incorporating into their envelopes proteins recognizing receptors specific for tumour cells (WO93/00103; WO92/14829). However, these constructs do not make it possible to obtain sufficient targeting, especially when the virus used encodes a toxic protein intended for the destruction of the cells.

The present invention provides an advantageous solution to these problems. It provides, indeed, vectors capable of directing the expression of a given gene selectively in the tumour cells. The present invention is based in particular on the demonstration that certain signals for control of transcription are active (or activated) specifically in the tumour cells, and that they can be used for the selective expression of heterologous genes. It also results from the demonstration that adenoviruses constitute particularly effective vectors for the transfer and expression of therapeutic genes in the tumour cells. In particular, adenoviruses have the advantage of not becoming integrated into the genome of the cells which they infect, of being maintained therein in a very stable manner, which makes it possible to obtain a lasting therapeutic effect and to have a very broad host range, which permits application to the treatment of cancers affecting any type of cells. Furthermore, recombinant adenoviruses can be obtained at high titres, which makes it possible to work at high multiplicities of infection, and to introduce several copies of the heterologous gene per cell. The invention is also based on the demonstration that adenovirus-type viruses are capable of incorporating heterologous sequences comprising such promoters, of transferring these sequences into the tumour cells, and of expressing desired genes under the control of specific signals directly in tumours.

A first subject of the invention therefore lies in a defective recombinant adenovirus containing a heterologous DNA sequence under the control of expression signals which are active specifically in tumour cells.

The subject of the invention is also the use of such a defective recombinant adenovirus for the preparation of a pharmaceutical composition intended for the treatment or the prevention of cancers.

The defective adenoviruses according to the invention are adenoviruses which are incapable of autonomously replicating in the target cell. Generally, the genome of the defective adenoviruses used within the framework of the present invention therefore lacks at least sequences necessary for the replication of the said virus in the infected cell. These regions can be either removed (completely or partially), or rendered non-functional, or substituted by other sequences and especially by the inserted gene. Preferably, the defective virus conserves, nevertheless, the sequences of its genome which are necessary for the encapsulation of the viral particles.

There are various serotypes of adenoviruses, whose structure and properties vary somewhat. However, these viruses are not pathogenic for man, and especially for non-immunosuppressed subjects. Among these serotypes, the use of type 2 or 5 human adenoviruses (Ad 2 or Ad 5) or of adenoviruses of animal origin (see Application FR 93 05954) is preferred within the framework of the present invention. Among the adenoviruses of animal origin which can be used within the framework of the present invention, there may be mentioned adenoviruses of canine, bovine,

murine (example: MAV1, Beard et al., Virology 75 (1990) 81), ovine, porcine, avian or alternatively simian (example: SAV) origin. Preferably, the adenovirus of animal origin is a canine adenovirus, more preferably a CAV2 adenovirus [Manhattan strain or A26/61 (ATCC VR800) for example].

Preferably, adenoviruses of human, canine or mixed origin are used within the framework of the invention.

As indicated above, the adenoviruses of the invention carry a heterologous DNA sequence. This heterologous DNA sequence permits the expression of a desired biological activity in tumour cells. Preferably, the heterologous DNA sequence comprises at least one gene chosen from a gene which is toxic for the infected cell, a gene whose expression makes it possible to at least partially inhibit cell division, or a gene encoding a lymphokine. The adenoviruses of the invention may, in addition, contain several of these sequences, so as to obtain, in some cases, a synergistic anti-tumour effect.

Among the genes which are toxic for the infected cell, there may be mentioned preferably the genes whose expression product confers on the cell sensitivity to a therapeutic agent. More preferably, the toxic gene is chosen from the thymidine kinase gene, whose expression product confers on mammalian cells sensitivity to certain therapeutic agents such as ganciclovir or acyclovir. The thymidine kinase of the herpes simplex virus is capable of phosphorylating nucleoside analogues such as acyclovir or ganciclovir. These modified molecules can be incorporated into a DNA chain undergoing elongation, which has as consequence the stopping of the DNA synthesis, resulting in the death of the cell (F. L. Moolten, Cancer Res. 46 (1986) 5276). This strategy thus makes it possible to remove specifically the cells expressing the suicide gene. Furthermore, the synthesis of DNA being the target of the toxicity, only the cells undergoing division are affected.

More preferably, the thymidine kinase gene of the human herpes virus (hTK HSV-1) is used within the framework of the present invention. The sequence of this gene has been described in the literature (see especially McKnight et al., Nucleic Acid. Res. 8 (1980) 5931).

It is also possible to use the cytosine deaminase gene, whose expression product confers on mammalian cells sensitivity to 5-fluorocytosine (5-FC). Moreover, among the toxic genes which can be used within the framework of the present invention, there may also be mentioned the genes whose expression product induces apoptosis of the infected cell.

Among the genes whose expression makes it possible to at least partially inhibit cell division, there may be mentioned more particularly tumour suppressor genes (or antioncogenes) or any active derivative thereof; antisense sequences or ribozymes, whose expression in the target cell makes it possible to at least partially inhibit the expression of genes promoting cell division.

Among the tumour suppressor genes which can be used within the framework of the present invention, there may be mentioned more particularly the p53 gene (Baker et al., Science 244 (1989) 217); the Rb gene (Friend et al., Nature 323 (1986) 643; Huang et al., Science 242 (1988) 1563); the rap 1A gene (Kitayama et al., Cell 56 (1989) 77); the DCC gene (Fearon et al., Science 247 (1990) 49), the k-rev2 and k-rev3 genes; or any other tumour suppressor genes described in the literature (cf. for example WO91/15580).

The heterologous DNA sequence may also contain an antisense sequence, whose expression in the target cell makes it possible to control the expression of genes promoting cell proliferation. This control may occur during

transcription, splicing of the premessenger, degradation of the messenger, its translation into protein, or post-translational modifications. Preferably, the heterologous DNA sequence contains a gene encoding an antisense RNA capable of controlling the translation of a target mRNA (EP 140 308). Among the antisense sequences which can be used within the framework of the invention, there may be mentioned more particularly any antisense sequence which makes it possible to reduce the levels of production of the oncogenes ras, myc, fos, c-erb B, and the like.

Among the genes encoding lymphokines, there may be mentioned more particularly genes encoding interleukins (preferably IL-1 to IL-3), interferons, tumour necrosis factors, colony-stimulating factors (G-CSF, M-CSF, GM-CSF, and the like), TGF-β, and the like. Moreover, the lymphokine-encoding gene generally comprises, upstream of the coding sequence, a signal sequence directing the synthesized polypeptide in the secretion pathways of the target cell. This signal sequence may be the natural signal sequence of the lymphokine, but it may also be any other functional signal sequence, or an artificial signal sequence. Such constructs make it possible in particular to increase the lymphokine levels in a very localized manner, and thus, in the presence of a tumour-specific antigen, to enhance the immune response against a particular type of tumour, which gives a particularly advantageous effect.

As indicated above, the heterologous DNA sequence is placed under the control of expression signals which are active specifically in tumour cells. In this way, the gene used is expressed and produces its effect only when the virus has indeed infected a tumour cell.

In a preferred embodiment of the invention, they are expression signals which are induced by or active in the presence of viruses responsible for or associated with tumours. Still more preferably, an expression signal inducible by the Epstein-Barr virus (EBV) or by the papilloma virus is used within the framework of the present invention.

The Epstein-Barr virus (EBV) is associated with two types of human cancers: Burkitt's lymphoma and cancer of the nasopharynx. The use of a recombinant adenovirus containing a toxic gene under the control of a promoter inducible by EBV makes it possible advantageously to express this toxic gene specifically in the tumour cells of the nasopharynx. In biopsies of cancers of the nasopharynx, only one nuclear antigen is regularly present, EBNA1, which is involved in the maintenance of the viral genome in the cells infected by EBV in the latent phase, and which transactivates the viral promoter BCR2. One particular subject of the invention therefore lies in the use, for the specific expression of a gene in nasopharynx cancer cells, of a sequence corresponding to EBNA1 (EBNA1-RE: EBNA1 "responsive element"). In particular, the invention relates to an adenovirus containing, as expression signal, a chimeric promoter containing a sequence corresponding to EBNA1 fused upstream of another viral promoter, the promoter of the terminal protein 1 (TP1) gene. The examples described in the present application indeed show that this chimeric promoter is inducible by EBNA1.

Papilloma viruses (especially the HPV 16 and 18 viruses) are responsible for 90% of cervical cancers in women and have been identified in pre-cancerous epithelial lesions (Riou et al., Lancet 335 (1990) 117). The product of the E6 gene leads to the formation of tumours by substantially decreasing the quantity of wild-type p53, an antioncogene, in HPV-positive cells (Wrede et al., Mol. Carcinog. 4 (1991) 171). The use of a recombinant adenovirus containing a toxic gene under the control of a promoter inducible by HPV

(for example protein E6) makes it possible advantageously to express this toxic gene specifically in the corresponding tumour cells.

They may also be expression signals which are inactive in normal cells and active in tumour cells. In particular, it is possible to use within the framework of the present invention the α-foetoprotein promoter (Alpert E., in Hepatocellular carcinoma, Okuda & Peters (eds), New York, 1976, 353) or the P3 promoter of IGF-II (Sussenbach et al., Growth Regulation 2 (1992) 1), which are active in adults, solely in hepatocarcinomas. It is also possible to use promoters induced by hormones in the case of hormone-dependent or hormone-associated tumours (breast or prostate tumours, for example).

In addition, these promoter sequences can be modified by addition of activating or regulatory sequences and the like.

The defective recombinant adenoviruses according to the invention can be prepared by any technique known to persons skilled in the art (Levrero et al., Gene 101 (1991) 195, EP 185 573; Graham, EMBO J. 3 (1984) 2917). In particular, they can be prepared by homologous recombination between an adenovirus and a plasmid carrying, inter alia, the heterologous DNA sequence. The homologous recombination occurs after cotransfection of the said adenoviruses and plasmid into an appropriate cell line. The cell line used should preferably (i) be transformable by the said elements, and (ii) contain the sequences capable of complementing the defective adenovirus genome part, preferably in integrated form in order to avoid risks of recombination. As an example of a cell line, there may be mentioned the human embryonic kidney line 293 (Graham et al., J. Gen. Virol. 36 (1977) 59) which contains especially, integrated in its genome, the left-hand part of the genome of an Ad5 adenovirus (12%). Strategies for constructing vectors derived from adenoviruses have also been described in Applications Nos. FR 93 05954 and FR 93 08596.

Then, the adenoviruses which have multiplied are recovered and purified according to conventional molecular biology techniques, as illustrated in the examples.

The present invention also relates to a pharmaceutical composition containing one or more defective recombinant adenoviruses as described above. Preferably, the pharmaceutical compositions of the invention contain a vehicle which is pharmaceutically acceptable for a formulation directly injectable into the tumours to be treated. This may be in particular isotonic sterile solutions, or dry, especially freeze-dried, compositions which, upon addition, depending on the case, of sterilized water or physiological saline, permit the preparation of injectable solutions. Direct injection into the tumour to be treated is advantageous since it makes it possible to concentrate the therapeutic effect at the level of the affected tissues. However, it is also possible to use pharmaceutical compositions formulated for topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous, intraocular or transdermal administration and the like.

The doses of defective recombinant adenovirus used for the injection can be adjusted according to various parameters, especially according to the mode of administration used, the pathology concerned, the gene to be expressed, or alternatively the duration of treatment desired. Generally, the recombinant adenoviruses according to the invention are formulated and administered in the form of doses of between 10^4 and 10^{14} pfu/ml, and preferably 10^6 to 10^{10} pfu/ml. The term pfu (plaque forming unit) corresponds to the infectivity of a virus solution, and is determined by infection of an appropriate cell culture, and measurement,

generally after 48 hours, of the number of plaques of infected cells. The techniques for determining the pfu titre of a viral solution are well documented in the literature.

The present invention thus offers a very effective means for the treatment of or prevention of cancers. It is most particularly suitable for the treatment of cancers of the nasopharynx or hepatocarcinomas.

In addition, this treatment may apply both to man and any animal such as ovines, bovines, domestic animals (dogs, cats and the like), horses, fish and the like.

The present invention will be more completely described with the aid of the following examples which should be considered as illustrative and non-limiting.

LEGEND TO THE FIGURES

FIG. 1: Representation of the vector pONT-tk

FIG. 2: Representation of the vector pONT-β-gal

GENERAL MOLECULAR BIOLOGY TECHNIQUES

The methods conventionally used in molecular biology, such as preparative extractions of plasmid DNA, centrifugation of plasmid DNA in caesium chloride gradient, agarose or acrylamide gel electrophoresis, purification of DNA fragments by electroelution, phenol or phenol-chloroform extraction of proteins, ethanol or isopropanol precipitation of DNA in saline medium, transformation in *Escherichia coli* and the like, are well known to persons skilled in the art and are widely described in the literature [Maniatis T. et al., "Molecular Cloning, a Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982; Ausubel F. M. et al. (eds), "Current Protocols in Molecular Biology", John Wiley & Sons, New York, 1987].

The pBR322- and pUC-type plasmids and the phages of the M13 series are of commercial origin (Bethesda Research Laboratories).

For the ligations, the DNA fragments can be separated according to their size by agarose or acrylamide gel electrophoresis, extracted with phenol or with a phenol/chloroform mixture, precipitated with ethanol and then incubated in the presence of phage T4 DNA ligase (Biolabs) according to the recommendations of the supplier.

The filling of the protruding 5' ends can be performed with the Klenow fragment of *E. coli* DNA polymerase I (Biolabs) according to the specifications of the supplier. The destruction of the protruding 3' ends is performed in the presence of phage T4 DNA polymerase (Biolabs) used according to the recommendations of the manufacturer. The destruction of the protruding 5' ends is performed by a controlled treatment with S1 nuclease.

Site-directed mutagenesis in vitro by synthetic oligodeoxynucleotides can be performed according to the method developed by Taylor et al. [Nucleic Acids Res. 13 (1985) 8749–8764] using the kit distributed by Amersham.

The enzymatic amplification of the DNA fragments by the so-called PCR technique [Polymerase-catalyzed-Chain Reaction, Saiki R. K. et al., Science 230 (1985) 1350–1354; Mullis K. B. and Faloona F. A., Meth. Enzym. 155 (1987) 335–350] can be performed using a DNA thermal cycler (Perkin Elmer Cetus) according to the specifications of the manufacturer.

The verification of the nucleotide sequences can be performed by the method developed by Sanger et al. [Proc. Natl. Acad. Sci. USA, 74 (1977) 5463–5467] using the kit distributed by Amersham.

EXAMPLES

Example 1.

Construction of the vector Ad-ONT-tk carrying the tk gene under the control of a chimeric promoter EBNA1-RE/TP1 (FIG. 1).

This example describes the construction of a recombinant adenovirus containing the herpes simplex virus thymidine kinase gene (tk) under the control of a promoter which is specifically active in the cells infected by the EBV virus (chimeric promoter EBNA1-RE/TP1).

1.1 Construction of the plasmid p7tk1

This example describes the construction of the plasmid p7tk1 containing the open reading frame of the tk gene of 1131 base pairs (ATG 114-116 and stop codon TGA 1242-1244), which-frame is inserted into a multiple cloning site.

The BglII-NcoI fragment containing the herpes simplex virus type 1 thymidine kinase (tk) gene was isolated from the plasmid pHSV-106 (marketed by Gibco BRL), repaired by the action of the klenow fragment and then inserted into the SmaI site of the plasmid pGEM7zf(+) (marketed by Promega). The SmaI and BglII sites are destroyed during this step, the NcoI site is conserved.

The plasmid obtained was designated p7tk1.

1.2. Construction of the plasmid pONT1

This example describes the construction of a plasmid containing a chimeric promoter consisting of a sequence required for the transactivation by the EBNA1 antigen and of the EBV virus TP1 promoter.

The EcoRI(7315)-SmaI(8191) fragment from the EBV virus was isolated from the strain B95-8. The complete sequence of the EBV virus has been described by Baer et al., (Nature 310 (1984) 207). This fragment contains the sequences required for the transactivation by the nuclear 1 antigen (EBNA1) (D. Reisman & B. Sugden, 1986, Molecular and Cellular Biology, vol. 6 pp. 3838–3846). This fragment was then fused to the NruI(166 241)-PstI(166 559) fragment from EBV B95-8 (the PstI site was digested with T4 polymerase), containing the TP1 promoter. The chimeric promoter thus obtained was then inserted into the multiple cloning site of the plasmid pBluescript II SK.

The plasmid obtained was designated pONT1.

1.3. Construction of the plasmid poNTtk

The plasmid pONTtk contains the herpes simplex virus thymidine kinase gene (tk) cloned into the plasmid p7tk1, under the control of the chimeric promoter EBNA1-RE/TP1 cloned into the plasmid pONT1.

To construct this plasmid, the BamHI-XhoI fragment of pONT1 which contains the chimeric promoter transactivated by EBNA-1 and EBNA-2, and the XhoI-ClaI fragment from p7tk1 which contains the tk open reading frame were cloned into the BamHI (478) and ClaI (4550) sites of the plasmid pAd.RSVβgal. The plasmid pAd.RSVβGal contains, in the 5'→3' orientation,

- the PvuII fragment corresponding to the left end of the Ad5 adenovirus containing: the ITR sequence, the replication origin, the signals for encapsulation and the enhancer E1A;
- the gene encoding β-galactosidase under the control of the RSV promoter (Rous sarcoma virus),
- a second fragment from the Ad5 adenovirus genome, which permits homologous recombination between the plasmid pAd.RSVβGal and the adenovirus d1324. The plasmid pAd.RSVβGal has been described by Stratford-Perricaudet et al. (J. Clin. Invest. 90 (1992) 626).

All the cloning sites are conserved. The plasmid obtained was designated pONTtk (FIG. 1)

1.4. Construction of the recombinant adenovirus Ad-ONT-tk

The vector pONTtk was linearized and cotransfected with a deficient adenoviral vector, into helper cells (line 293) providing in trans the functions encoded by the adenovirus E1 regions (E1A and E11B).

More precisely, the adenovirus Ad-ONT-tk was obtained by homologous recombination in vivo between the mutant adenovirus Ad-d1324 (Thimmappaya et al., Cell 31 (1982) 543) and the vector pONTtk, according to the-following procedure: the plasmid pONTtk, linearized with XmnI, and the adenovirus d1324, linearized with the enzyme ClaI, were cotransfected into the line 293 in the presence of calcium phosphate, so as to allow the homologous recombination. The recombinant adenoviruses thus generated were selected by plate purification. After isolation, the DNA from the recombinant adenovirus was amplified in the cell line 293, which leads to a culture supernatant containing the unpurified recombinant defective adenovirus having a titre of-about 10^{10} pfu/ml.

The viral particles are generally purified by caesium chloride gradient centrifugation according to known techniques (see especially Graham et al., Virology 52 (1973) 456). The adenovirus Ad-ONT-tk can be preserved at −80° C. in 20% glycerol.

Example 2.

Construction of the vector Ad-ONT-βgal

This example describes the construction of a recombinant adenovirus containing the *E. coli* beta-galactosidase gene (βgal) under the control of a promoter which is specifically active in the cells infected by the EBV virus (chimeric promoter EBNA1-RE/TP1).

2.1. Construction of the plasmid pONT-βgal

The XbaI-(HindIII) fragment from the plasmid pONT1 which contains the chimeric promoter transactivated by EBNA-1 and EBNA-2 and the fragment (StuI)-KpnI from the plasmid pAd.RSVβgal which contains the β-galactosidase gene were cloned into the XbaI (484) and KpnI (4520) sites of the plasmid pAd.RSVβgal. The HindIII and StuI sites are destroyed during this step. The plasmid obtained was designated pONT-βgal (FIG. 2).

2.2. Construction of the-Ad-OTT-βgal adenovirus

The vector pONT-βgal obtained in Example 2.1, was used, by homologous recombination according to the procedure described in Example 1.4., to prepare a recombinant adenovirus containing the *E. coli* beta-galactosidase gene (βgal) under the control of the chimeric promoter EBNA1-RE/TP1. The Ad-ONT1-βgal adenovirus thus obtained can be preserved at −80° C. in 20% glycerol.

Example 3.

Construction of the vector pONT-CAT

This example describes the construction of a vector comprising the chloramphenicol acetyltransferase (CAT) gene under the control of a promoter which is specifically active in the cells infected by the EBV virus (chimeric promoter EBNA1-RE/TP1).

3.1. Construction of the vector

The EBV TP1-promoter was isolated in the form of the EBV NruI(166241)-PstI(166559) fragment. This fragment was then fused to the CAT gene, and inserted, in the form of an NruI-BamHI fragment, into the plasmid pGem7ZF (Promega). The resulting plasmid was designated pTP1-CAT. The NruI-BamHI fragment from the plasmid pTP1-CAT was then fused, downstream of the EBV strain B95-8 EcoRI(7315)-SmaI(8191) fragment, containing the sequences required for the transactivation by EBNA1 (cf. Example 1.2.). The fragment obtained, comprising the CAT

gene under the control of the chimeric promoter EBNA1-RE/TP1, was inserted into the EcoRI and BamHI sites of the plasmid pBluescript SK in order to generate the plasmid pONT-CAT. A plasmid was also constructed from which the elements for responding to the EBNA2 antigen of the TP1 promoter were deleted. For that, the TP1 promoter was isolated in the form of the EBV NruI(166375)-PstI(166559) fragment. This plasmid was designated pOST-CAT.

3.2. Activity in vitro

This example demonstrates that the constructs described above are induced specifically by antigens of the Epstein-Barr virus.

The vectors pONT-CAT, pOST-CAT and pTP1-CAT were transfected by electroporation into an EBV$^-$ B lymphocyte line (DG75 cells), either alone or in the presence of vectors for expressing the viral antigens EBNA1, EBNA2 or EBNA1+EBNA2. 48 hours after the transfection, the cells were lysed by freezing/thawing, the cell debris removed and then the extracts obtained were standardized depending on the quantity of proteins. The CAT activity was then assayed in these extracts by enzymatic assay. The results obtained are the following:

	Alone	+EBNA2	+EBNA1	+EBNA1/A2
pTP1-CAT	1	35	2.5	17
pONT-CAT	1	16	34	136
pOST-CAT	1	1.4	19.5	22.5

These results show clearly that the ONT promoter is specifically active in the presence of the antigens EBNA1 and EBNA2, and that it induces a very high expression of the CAT gene.

We claim:

1. A replication defective recombinant adenovirus comprising a heterologous DNA sequence under the control of an expression signal which is inducible by the Epstein-Barr virus (EBV) or by a papilloma virus antigen.

2. An adenovirus according to claim 1, wherein the expression signal is activated by the EBNA1 antigen.

3. An adenovirus according to claim 2, wherein the expression signal consists of a chimeric promoter compris-

ing a sequence which is activated by EBNA1 antigen fused upstream of a viral promoter

4. An adenovirus according to claim 1, lacking regions of its genome which are required for replication in a target cell.

5. An adenovirus according to claim 4, wherein said adenovirus is a type Ad5 human adenovirus or a type CAV-2 canine adenovirus.

6. An adenovirus according to claim 1, wherein the heterologous DNA sequence comprises a gene which encodes a product toxic in a cell infected by said adenovirus.

7. An adenovirus according to claim 6, wherein said product renders said cell sensitive to a therapeutic agent.

8. An adenovirus according to claim 7, wherein the gene is the thymidine kinase gene and the therapeutic agent is ganciclovir or acyclovir.

9. An adenovirus according to claim 1, wherein the heterologous DNA sequence comprises a gene which encodes a product effective to inhibit cell division.

10. An adenovirus according to claim 9, wherein the gene is selected from the group consisting of tumour suppressor genes, antisense sequences and ribozymes.

11. An adenovirus according to claim 6, wherein the heterologous DNA sequence comprises a gene whose expression product induces apoptosis of a cell infected by said adenovirus.

12. A composition comprising a replication defective recombinant adenovirus according to claim 1 and an acceptable carrier.

13. A composition according to claim 12, in injectable form.

14. The adenovirus of claim 4, wherein the viral promoter is the terminal protein 1 (TP1) gene promoter.

15. An isolated cell comprising the adenovirus of claim 1.

16. The composition of claim 12 comprising from 10^6 to 10^{10} pfu/ml of replication defective recombinant adenoviruses.

* * * * *

US005837531A

United States Patent [19]

Dedieu et al.

[11] Patent Number: 5,837,531

[45] Date of Patent: Nov. 17, 1998

[54] RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS

[75] Inventors: **Jean-François Dedieu**, Paris; **Aude Le Roux**, Chevilly La Rue; **Michel Perricaudet**, Ecrosnes, all of France

[73] Assignee: **Rhone-Poulenc Rorer S.A.**, Antony Cedex, France

[21] Appl. No.: **646,246**

[22] PCT Filed: **Nov. 7, 1994**

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§ 371 Date: **May 13, 1996**

§ 102(e) Date: **May 13, 1996**

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PCT Pub. Date: **May 26, 1995**

[30] **Foreign Application Priority Data**

Nov. 18, 1993 [FR] France 93 13766

[51] **Int. Cl.**[6] **C12N 15/63**

[52] **U.S. Cl.** **425/320.1**; 436/172.3; 436/69.1; 436/91.4; 436/325; 514/44

[58] **Field of Search** 514/44, 49; 435/172.3, 435/91.4, 69.1, 325, 320.1; 935/16, 23, 24, 34, 42, 57, 62, 70

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,939,088	7/1990	Young et al.	435/320.1
5,194,601	3/1993	Sugden et al.	435/320.1

FOREIGN PATENT DOCUMENTS

WO 92/05262 4/1992 WIPO .

WO 93/19191 3/1993 WIPO .

OTHER PUBLICATIONS

Stefan Karlsson (1991) Blood, vol. 78, No. 10: pp. 2481–2492.

Dong et al. (1996) Human Gene Therapy 7: 319–331.

Morsy et al. (Nov. 17, 1993) JAMA, vol. 270, No. 19, pp. 2338–2345.

Ronald Crystal (1995) Science, vol. 270, pp. 404–409.

Coghlan (25 Nov., 1995) New Scientist pp. 14–15, vol. 149.

Marshall (Aug. 1995) Science, vol. 269, pp. 1050–1055.

Marshall (Dec. 1995) Science, vol. 270, p. 1751.

Orkin & Moltulsky (Dec. 7, 1995) NIH Report on Gene Therapy.

Sugden et al. (1989) Journal of Virology, pp. 2644–2649.

Zimber–Strobl et al. (1991) Journal of Virology, pp. 415–423.

Perricaudet et al. (1992) Ann Oncol., vol. 3, suppl. 5, p. 135.

Mastrangelo et al (1993) The Journal of Clinical Investigation, vol. 91, pp. 225–234.

Jean Marx (1993) Science, vol. 259, pp. 760–761, Jul. 30, 1996.

Kozarsky et al. (1993) Current Opinion in Genetics and Development, 3, pp. 499–503, Jul. 30, 1996.

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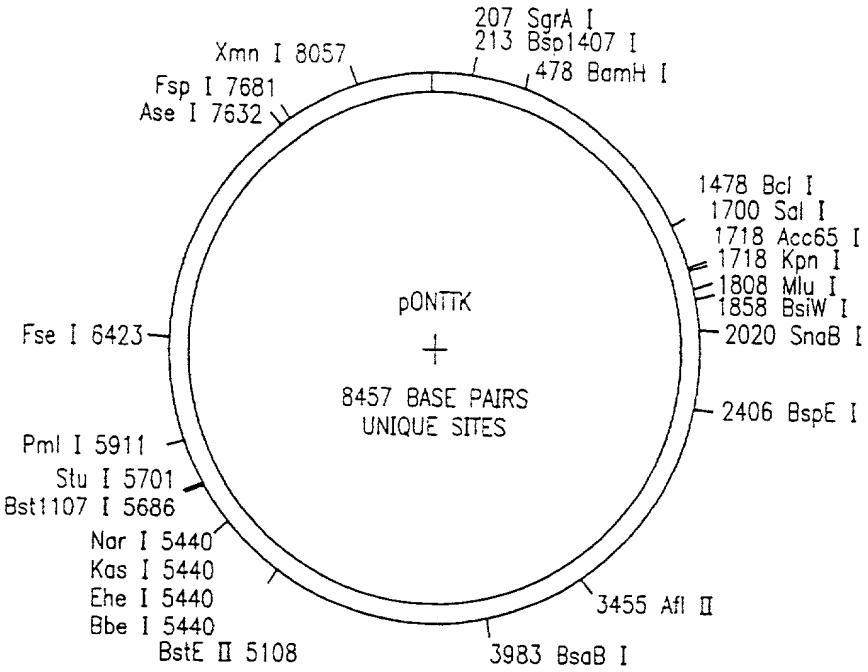
Assistant Examiner—Dave Trong Nguyen

[57] **ABSTRACT**

The invention concerns recombinant viruses comprising a heterologous DNA sequence under the control of expression signals specifically active in tumour cells, and their preparation and use in the treatment and prevention of cancers

16 Claims, 1 Drawing Sheet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Bbe I 5440
Ehe I 5440
Kas I 5440
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Bst1107 I 5686
Stu I 5701
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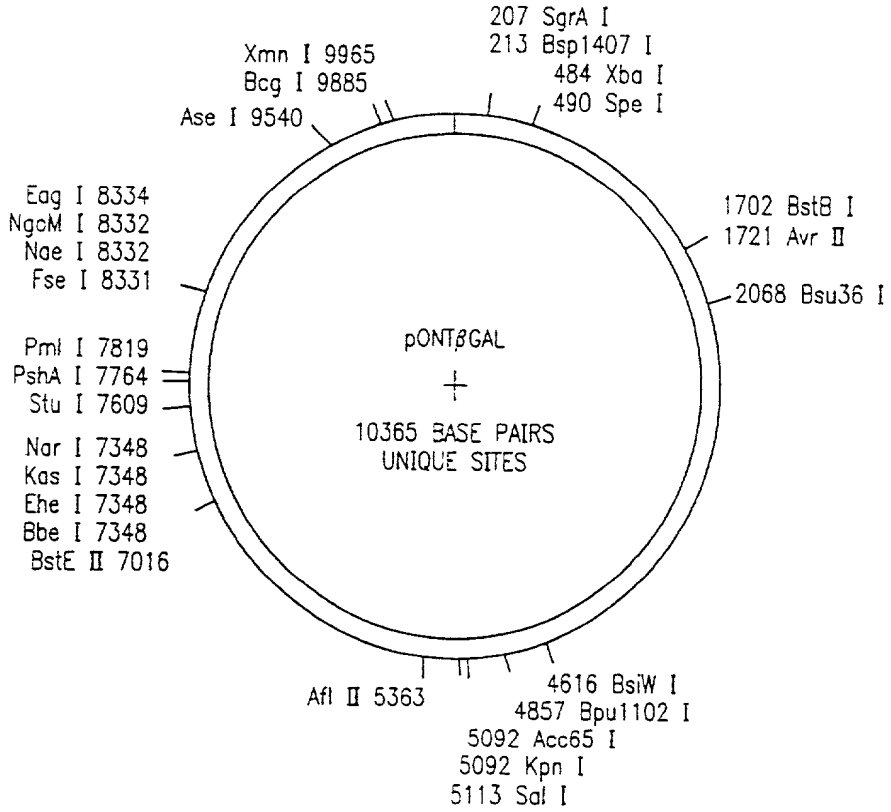
Xmn I 8057
pONTK
8457 BASE PAIRS
UNIQUE SITES

FIG. 1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213 Bsp1407 I
484 Xba I
490 Spe I
1702 BstB I
1721 Avr II
2068 Bsu36 I
4616 BsiW I
4857 Bpu1102 I
5092 Acc65 I
5092 Kpn I
5113 Sal I
Afl II 5363
BstE II 7016
Bbe I 7348
Ehe I 7348
Kas I 7348
Nar I 7348
Stu I 7609
PshA I 7764
Pml I 7819
Fse I 8331
NgoM I 8332
Nae I 8332
Eag I 8334
Ase I 9540
Bcg I 9885
Xmn I 9965
pONTβGAL
10365 BASE PAIRS
UNIQUE SITES

FIG. 2

PATENT
Attorney Docket No. 03804.1129-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of: U.S. Patent No. 5,837,531	)	
Issued: November 17, 1998	)	
Inventors: Jean-Francois DEDIEU et al.	)	Group Art Unit: Unassigned
Reissue Serial No.: Unassigned	)	Examiner: Unassigned
Filing Date of Reissue Appln.: August 31, 2000	)	
For: RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS	)	

ATTN.: BOX REISSUE
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

REISSUE DECLARATION UNDER 37 C.F.R. § 1.175

We, Jean-Francois DEDIEU, Aude LE ROUX, and Michel PERRICAUDET, all citizens of France, with residences and post office addresses as listed below, do hereby state and declare as follows:

1. We believe we are the original joint inventors of the subject matter which is described and claimed in United States Patent No. 5,837,531, granted on November 17, 1998, and for which a reissue patent is sought on the invention entitled, "RECOMBINANT ENDOVIRUSES FOR GENE THERAPY IN CANCERS."

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2. We hereby claim the benefit under 35 U.S.C. § 119(a) of the foreign application FR93/13766 filed in France on November 18, 1993. A certified copy of FR93/13766 is of record in the file of U.S. Application Serial No. 08/646,246, which gave rise to United States Patent No. 5,837,531.

3. We hereby state that we have reviewed and understand the contents of the above-identified specification, including the original patent claims, and the claims in the Preliminary Amendment filed herewith.

4. We acknowledge the duty to disclose information that is material to the examination of this reissue application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

5. We believe that U.S. Patent No. 5,837,531 is at least partially inoperative or invalid by reason of defective claim language.

6. An error which is a statutory basis for reissue is the lack of direct antecedent basis for the term "the EBNA 1 antigen" in claim 2. The lack of direct antecedent basis for the term "the EBNA 1 antigen in claim 2 may render the claim vague and indefinite under 35 U.S.C. § 112, second paragraph.

7. The above-described error and all other errors corrected in this reissue application arose without any deceptive intent.

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8. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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PATENT
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Inventors: Jean-Francois DEDIEU et al.	)	Group Art Unit: Unassigned
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8. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Residence		Citizenship
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202-408-4000

PATENT
Attorney Docket No. 03804.1129-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of:
U.S. Patent No. 5,837,531

Issued: November 17, 1998

Inventors: Jean-Francois DEDIEU et al. — Group Art Unit: Unassigned

Reissue Serial No.: Unassigned — Examiner: Unassigned

Filing Date of Reissue Appln.: August 31, 2000

For: RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS

REISSUE APPLICATION FEE TRANSMITTAL FORM

ATTN.: BOX REISSUE
Assistant Commissioner for Patents
Washington, D.C. 20231

Claims as Filed - Part 1								
Claims in Patent	For	Number Filed In Reissue Application	(3) Number Extra	Small Entity		Other Than a Small Entity		
				Rate	Fee	Rate		Fee
(A) 16	Total Claims (37 CFR 1.16(j))	(B) 16	0 =	x$___=		or	x$18 =	-0-
(C) 1	Independent Claims (37 CFR 1.16(i))	(D) 1	0 =	x$___=			x$ 78 =	-0-
				Basic Fee (37 CFR 1.16(h))	$___	OR		$ 690.00
				Total Filing Fee	$___			$ 690.00

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Claims as Amended - Part 2									
	(1) Claims Remaining After Amendment		(2) Highest Number Previously Paid For	(3) Extra Claims Present	Small Entity			OtherThan a Small Entity	
					Rate	Fee		Rate	Fee
Total Claims (37 CFR 1.16(j))	16	MINUS	16	= 0	x$____=		or	x$ 18 =	-0-
Independent Claims (37 CFR 1.16(i))	1	MINUS	1	= 0	x$____=			x$ 78 =	-0-
Total Additional Fee						$	OR		$-0-

A check is enclosed for **$690.00** to cover the Basic Filing Fee.

If there are any fees due under 37 C.F.R. §§ 1.16 or 1.17 which are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: _____
Timothy B. Donaldson
Reg. No. 43,592

Dated: August 31, 2000

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WASHINGTON, DC 20005
202-408-4000

PATENT NUMBER: 5837[illegible]1 ISSUE DATE: 11/17/98
SERIAL NUMBER: [illegible] FILING DATE: [illegible]
TITLE: RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS
APPLICANTS: DEDIEU, JEAN-FRANCOIS; LE ROUX, ALINE
PERRICAUDET, MICHEL

REEL: [illegible] FRAME: [illegible] DATE RECORDED: [illegible] NUMBER OF PAGES: [illegible]
ASSIGNOR: DEDIEU, JEAN-FRANCOIS
EXC DATE: [illegible]
LE ROUX, ALINE
EXC DATE: 04/[illegible]
PERRICAUDET, MICHEL
EXC DATE: [illegible]
ASSIGNEE: RHONE-POULENC RORER, S.A.
20 AVENUE RAYMOND ARON
92165 ANTONY CEDEX, FRANCE
BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

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04 Rec'd PCT/PTO 13 MAY 1996

06-13-1996

FORM PTO-1595
1-31-92
Docket No.EX93019-US

RECORDATION

100210129

COMMERCE
Jemark Office

To the Honorable Commissioner of Patents and Trademarks. Please record the attached original documents or copy thereof

1 Name of conveying party(ies).

DEDIEU, Jean-Francois
LE ROUX, Aude
PERRICAUDET, Michel

Additional name(s) of conveying party(ies) attached? [ ]yes [X]no

3 Nature of Conveyance.
[X]Assignment [ ]Merger
[ ]Security Agreement [ ]Change of Name
[ ]Other
Execution Date (of Assignment). April 25, 1996

2. Name and address of receiving party(ies)

Rhône-Poulenc Rorer S A
20 Avenue Raymond Aron
92165 Antony Cedex
FRANCE

Additional name(s) & address(es) attached? [ ]Yes [X]No

4. Application number(s) or patent number(s)

If this document is being filed together with a new application, the execution date of the application is.

A. Patent Application No (s)
U S National Stage of PCT/FR94/01284

B Patent No (s)

Additional numbers attached? [ ] Yes [X] No

5 Name and address of party to whom correspondence concerning document should be mailed:

Name Julie K Smith
Internal Address Rhône-Poulenc Rorer Inc
Legal-Patents, #3C43

Street Address: P O Box 5093

City Collegeville State PA ZIP 19426-0997

6 Total number of applications and patents involved [1]

7 Total fee (37 CFR 3.41):..... $ 40.00

70029 U.S. PTO

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8 Deposit account number
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Julie K Smith
Name of Person Signing

Signature

13 May 1996
Date

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ASSIGNMENT AND AGREEMENT

For value received and intending to be legally bound, we, the undersigned joint inventors (Assignors), sell, assign and transfer to RHÔNE-POULENC RORER S.A. (Assignee), having a principal place of business at 20 Avenue Raymond Aron, 92165 Antony Cedex, FRANCE, and its successors, assigns and legal representatives, the entire right, title and interest in and to our invention relating to

RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS

as disclosed and claimed in and to the application for United States Letters Patent therefor, assigned Attorney Docket No. **EX93019 US** filed on _____ as Serial No. _____ as the U.S. national stage of PCT Serial No. **FR94/01284** filed in the **French Receiving Office** on **7 November 1994**, and under any and all Letters Patent which may be granted therefor, and all right, title and interest in and to every patent application filed or to be filed on said invention in the United States, including renewals, revivals, continuations, divisions and any substitute applications of said United States application, and any and all patents which may issue thereon, and any reissues and extensions of the same.

We authorize and request competent authorities to grant and issue any and all patents on said invention to said assignee or its successors, assigns and legal representatives, or to such nominees as said assignee may designate.

We agree that, when requested, we will, without charge to said assignee but at its expense, sign all papers, take all rightful oaths, and do all acts which may be necessary, desirable or convenient for securing and maintaining patents for said invention in any and all countries and for vesting title thereto in said assignee, its successors, assigns, legal representatives or nominees.

We covenant with said assignee, its successors, assigns and legal representatives, that the rights and property herein conveyed are free and clear of any encumbrance, and that we have full right to convey the same as herein expressed.

Signed at Villejuif FRANCE on this 25th day of April, 1996.
(City, State and Nation)

Signature: Jean-François Dedieu

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April, 1996.

Signature: Aude le Roux

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Signed at Villejuif, FRANCE on this 25th day of
(City, State and Nation)
April, 1996.

Signature: Michel PERRICAUDET

Inventor's Name: Michel PERRICAUDET
Residential Address: 31, rue de Chartres
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FRANCE

PATENT
Attorney Docket No. 03804.1129-00000
RPR Docket No. EX93019-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of: U.S. Patent No. 5,837,531	)	
Inventors: Jean-Francois DEDIEU et al.	)	
Reissue Serial No.: Unassigned	)	Group Art Unit: Unassigned
Reissue Application Filed: August 31, 2000	)	Examiner: Unassigned
For: RECOMBINANT ADENOVIRUSES FOR GENE THERAPY IN CANCERS	)	

ATTN.: BOX REISSUE
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97(a)

Pursuant to 37 C.F.R. §§ 1.56 and 1.97(a), Applicants bring to the attention of the Examiner the documents listed on the attached Form PTO-1449. This Information Disclosure Statement is being filed before the mailing date of a first Office Action on the merits for the above-referenced reissue proceeding.

Copies of the listed documents were previously submitted to or cited by the Examiner in prior application Serial No. 08/646,246, filed May 13, 1996, now U.S. Patent No. 5,837,531, which was a § 371 National Phase filing of International Application No. PCT/FR94/01284, filed Nov. 7, 1994. Copies of the listed documents are not attached pursuant to 37 C.F.R. § 1.98(d).

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This submission does not represent that a search has been made or that no better art exists and does not constitute an admission that each or all of the listed documents are material or constitute "prior art." If the Examiner applies any of the documents as prior art against any claim in the application and Applicants determine that the cited documents do not constitute "prior art" under United States law, Applicants reserve the right to present to the office the relevant facts and law regarding the appropriate status of such documents.

Applicants further reserve the right to take appropriate action to establish the patentability of the disclosed invention over the listed documents, should one or more of the documents be applied against the claims of the present application.

If there is any fee due in connection with the filing of this Statement, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: _____
Timothy B. Donaldson
Reg. No. 43,592

Date: August 31, 2000

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INFORMATION DISCLOSURE CITATION

Atty. Docket No.:	03804.1129-00000		
Applicant:	Jean-Francois DEDIEU et al.	**Serial No.:**	Unassigned
Filing Date:	August 31, 2000	**Group Art Unit:**	Unassigned

U.S. PATENT DOCUMENTS

Examiner's Initials*		Document Number	Date	Name	Class	Sub Class	Filing Date (if appropriate)
	1	4,939,088	07/1990	Young et al.	435	320.1	
	2	5,194,601	03/1993	Sugden et al.	435	320.1	
	3	5,672,344	09/30/97	Kelley et al.	424	172.1	

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Sub Class	Translation Yes or No
	4	WO 92/05262	4/1992	WIPO			
	5	WO 93/19191	3/1993	WIPO			

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

	6	**Clayman et al.,** *"Adeno p53 Gene Transfer in a Phase I/II Trial of Patients with Advanced Recurrent Head and Neck Squamous Carcinoma,"* Soc. for Biol. Therapy, Ann. Meeting, Abstract (1996)
	7	**Clayman et al.,** *"Adenovirus Mediated p53 Gene Transfer in a Phase I Trial of Patients with Advanced Recurrent Head and Neck Squamous Carcinoma,"* ASCO Annual Meeting, Abstract (1997)
	8	**Clayman et al.,** *"Adenovirus Mediated p53 Gene Transfer in Patient with Advanced Recurrent Head and Neck Squamous Carcinoma,"* AACR Annual Meeting, Abstract
	9	**Clayman et al.,** *"Gene Therapy for Head and Neck Cancer: Comparing the Tumor Suppressor Gene p53 and a Cell Cycle Regulator WAFI/CIP1 (p21),"* Arch. Otolaryngol. Head Neck Surgery, Vol. 122, pp. 489-493 (1996)

Examiner:	**Date Considered:**
* Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

	10	**Coghlan**, *"New Scientist,"* Vol. 149, pp. 14-15 (1995)
	11	**Crystal,** *"Transfer of genes to humans: early lessons and obstacles to success,"* Science Vol. 270, pp. 404-409 (1995)
	12	**Dong et al.,** *"Systematic analysis of repeated gene delivery into animal lungs with a recombinant adenovirus,"* Human Gene Therapy Vol. 7, pp. 319-331 (1996)
	13	**Karlsson,** *"Treatment of genetic defects in hematopoietic cell function by gene transfer,"* Blood, Vol. 78, No. 10, pp. 2481-2492 (1991)
	14	**Kozarsky et al.,** *"Gene therapy: adenovirus vectors,"* Current Opinion on Genetics and Development, Vol. 3, pp. 499-503 (1996)
	15	**Marshall,** *"Gene therapy's growing pains,"* Science, Vol. 269, pp. 1050-1055 (1995)
	16	**Marshall,** *"Less hype, more biology needed for gene therapy,"* Science, Vol. 270, p. 1751 (1995)
	17	**Marx,** *"Cell death studies yield cancer clues,"* Science, Vol. 259, pp. 760-761 (1996)
	18	**Mastrangeli et al.,** *"Diversity of airway epithelial cell targets for in vivo recombinant adenovirus-mediated gene transfer,"* The Journal of Clinical Investigation, Vol. 91, pp. 225-234 (1993)
	19	**Morsy et al.,** *"Progress toward human gene therapy,"* JAMA, Vol. 270, No. 19, pp. 2338-2345 (1993)
	20	**Orkin & Moltulsky**, NIH Report on Gene Therapy, (Dec. 7, 1995)
	21	**Perricaudet et al.**, Ann. Oncol., Vol. 3, Suppl. 5, p. 135 (1992)
	22	**Roth et al.,** *"Retrovirus-Mediated Wild-Type p53 Gene Transfer to Tumors of Patients with Lung Cancer,"* Nature Medicine, Vol. 2, pp. 985-991 (1996)
	23	**Sugden et al.,** *"A promoter of Epstein-Barr virus that can function during latent infection can be transactivated by EBNA-1, a viral protein required for viral DNA replication during latent infection,"* Journal of Virology, pp. 2644-2649 (1989)
	24	**Swisher et al.,** *"Persistant Transgene Expression Following Repeated Injections of a Recombinant Adenovirus Containing the p53 Wild-Type Gene in Patients with Non-Small Cell Lung Cancer,"* AACR Annual Meeting, Abstract
	25	**Swisher et al.,** *"Adenoviral Mediated p53 Gene Transfer in Patients with Advanced Non-Small Cell Lung Cancer (NSCLC),"* ASCO Annual Meeting, Abstract (1997)
	26	**Zimber-Strobl et al.,** *"Epstein-Barr virus nuclear antigen 2 activates transcription of the terminal protein gene,"* Journal of Virology pp. 415-423 (1991)

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